The Examiner first objected to the specification because of the numbering of the amino acids on page 7 of the specification. That reference has been corrected to be consistent with the rest of the specification.

A separate change to the specification was made in the paragraph bridging pages 5 and 6 of the application, to try to help with the issue of the numbering of the amino acids. At the bottom of page 5 of the specification as filed, the applicant incorporated by reference the Lee et al. article from *Biochemistry*. The numbering of amino acids used in the specification of the application was the numbering convention used in the Lee et al. paper. If the Examiner will refer to the Lee et al. paper, the Examiner will see that the N-terminal methionine in Lee is denominated "0" rather than "1." That is the reason for the difference in numbering between the amino acid sequence listing in this application and the numbering used in the specification. The PTO sequence program automatically assigns the N-terminal methionine to be "1." The insertion to this paragraph in the specification is just to make it clear that the nomenclature used in the specification was that described in Lee, and to explain the inconsistency with the sequence listing. Since Lee was incorporated by reference in the application, and because those of ordinary skill in the art are well aware of the fact that N-terminal methionines are considered a special case amino acid residue, this addition to the specification does not represent new matter whatsoever, but instead represents a clerical clarification to make the subject matter clearer.

The Examiner rejected the claims under Section 112, second paragraph, for indefiniteness. The applicant has made changes to the claims above intended to make it clear that the substitution in the ribonuclease inhibitor is for a cysteine residue which is adjacent to another cysteine residue. The applicant understands the Examiner's concerns about definiteness and have amended Claims 1, 9 and 11 so as to be more clear in this regard. While this change does make the language more definite, it is not believed that this modification changes the scope of the claims. It is, however, believed that this change cures the indefiniteness problem cited by the Examiner.

Similarly, the objection to Claims 2 and 10 is believed cured by the language imported into the specification, which makes it clear that the applicant is counting the cysteines which appear at positions 94 and 95, 328 and 329, using the nomenclature of Lee et al.

The other two rejections in the Office Action are under Section 112, first paragraph. As understood by the applicant, this rejection is premised on the fact that the applicant's claims are too broad in that they extend beyond human ribonuclease inhibitor and extend beyond the amino acid substitutions described in the specification. If this understanding is correct, the applicant does not understand the rejection of Claim 10, which encompasses only human ribonuclease inhibitor and which specifically recites a substituted residue at one of amino acid positions 94 and 95, 328 and 329. As shown in the examples contained in this application, substitutions for the cysteine at any of these positions result in ribonuclease inhibitors that are more resistant to oxidation. Accordingly, it is thought that perhaps the rejection is because the Examiner believes that the only evidence is for a substitution to alanine. If that is correct, the applicant has submitted a new Claim 15 which ought to be in allowable form and not subject to this rejection, even if the rejection is maintained after this response.

However, the applicant believes that reasoning limiting the applicant in following the argument of this rejection would be unduly restrictive. In response to both this rejection, and the following one, the applicant respectfully notes that the applicant has provided a thesis as to why ribonuclease inhibition has its well-known susceptibility to oxidation. The thesis is that adjacent cysteine residues are the most likely to be oxidized to form disulfide bonds, which do not natively occur in the tertiary structure of the native protein in nature, but which, when they occur, make the protein less biologically effective to inhibit ribonuclease activity. This thesis is clearly stated in the specification. There is a drawing figure (Fig. 2) illustrating what occurs and explaining how that event is relevant to the inhibiting activity of the ribonuclease inhibitor. The applicant then went on to conduct the experiments described in the examples, based upon the hypothesis that the applicant proposed, and demonstrated that the thesis was correct. Thus the experimental data provided in this application is not fortuitous or accidental data for which an explanation must be found in hindsight. This data was recreated following the applicant's perception that the reason for the oxidative instability of ribonuclease inhibitor was formation of disulfide bridges between adjacent cysteine residues in the protein. The applicant's data have verified that hypothesis.

Since the reason for the susceptibility of the native molecule to oxidation is the formation of the disulfide bridges between adjacent cysteine residues, it therefore follows

quite logically and clearly that virtually any substitution for either of the cysteine residues that are paired in that fashion would increase the resistance to oxidation of the molecule. Only cysteine amino acids can form disulfide bridges with adjacent cysteines. The alanines substituted in examples described in the specification in no way change the function or activity of the protein. As shown in the three-dimensional structure of the molecules submitted herewith, the portion of the molecule in which the adjacent disulfides exist is not in any portion of the molecule which comes in contact with the ribonuclease enzyme, during the binding that inhibits the functioning of the ribonuclease.

Thus, the applicant has provided a clear theoretical explanation as to why modifications of this category are effective and further has provided data demonstrating experimental results that fit precisely within that theoretical prediction. Note also that the applicant has provided, by virtue of an amino acid best fit analysis provided in Figure 7, a clear indication as to where similar modifications can be made in other non-human ribonuclease inhibitors to achieve a similar result. Thus a full written description of several variants of the application is contained in the specification.

For that reason it is submitted that the claims are fully enabled to the breadth of the subject matter of Claims 1 to 10, and that the full scope of such claims is reasonably conveyed to one of ordinary skill in the art.

Contrary to what the Examiner states on page 7, the applicant believes that he has demonstrated that the regions of the protein structure which are proposed to be modified can be modified without effecting ribonuclease inhibitor activity. If one reviews the amino acid sequence of the molecule, and reviews the three-dimensional structure as set forth in Figure 1, one can see that the modifications are in places on the protein structure that are not involved in binding the ribonuclease.

Also, the applicant believes that he has demonstrated that the ribonuclease inhibitor can be modified successfully, as demonstrated in the experimental data provided herewith.

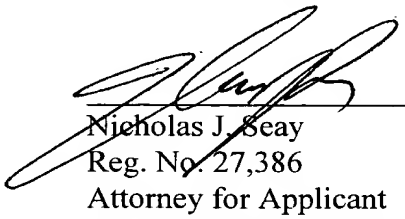
Additionally, the applicant believes that he has provided a rational and predictable scheme for modifying amino acid residues with an expectation of obtaining the desirable biological function. The applicant has identified the type of amino acids to be modified, i.e., either one of adjacent cysteine residues and has demonstrated that such modifications of those residues to an amino acid which will not form a disulfide bond will have the desired effect. A

rational explanation for this phenomenon is also provided in the specification, i.e., increasing the resistance to oxidation by preventing the formation of disulfide bonds which disrupt the three dimensional structure of the molecule. Therefore, the specification does provide clear guidance as to what modifications within the class of molecules described in the claims would be successful. The claims also have a scope which closely corresponds to the theoretical basis of the effect predicted by the applicant. Accordingly, it is not believed that the claims are too broad under 35 U.S.C. §112, and it is requested that this rejection be reconsidered.

Lastly in the Office Action was a rejection under 35 U.S.C. §102 to Chen et al. Chen does describe a modification of a cysteine residue present at residue 408 in the sequence of the ribonuclease inhibitor. The applicant understands that this rejection was made because the Examiner felt that the applicant had not clearly recited in the claims as filed that the substituted cysteine residue in question had to be adjacent to another cysteine residue. Since the claims have been amended to make it clear that this is a requirement for the substitution to make the mutant protein of the present invention, it is believed that this rejection under §102 is no longer relevant. In addition, it is believed that the rejection does not make the claims obvious since nothing in Chen suggests any motivation or likelihood of success in doing what the applicant here has done.

Accordingly, reconsideration of the merits of this patent application is respectfully requested. A petition for extension of time is submitted herewith so that this response will be considered as timely filed.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Applicant: Ronald T. Raines

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Examiner: R. Hutson

Title: OXIDATION-RESISTANT RIBONUCLEASE INHIBITOR

File No.: 960296.95360

In the Specification:

Please insert the following new paragraph for the paragraph which previously appeared in the specification starting on page 5, line 31 and extending onto page 6, line 7.

An illustration of the three dimensional structure of the human ribonuclease inhibitor is illustrated in Fig. 1. The sequence of the ribonuclease inhibitor can be found in Lee et al. Biochemistry 27:8545-8553 (1988), the disclosure of which is hereby incorporated by reference. From both Figure 1 and the sequence of the protein, it can be readily seen that some of the cysteine residues are located adjacent to each other. The amino acid residues at positions numbered 95 and 96 and 328 and 329 in the human RI sequence as numbered in Lee et al. are all cysteines. It was theorized that these cysteine residues would be the most likely to be oxidized to form disulfide bonds which would interfere with the biological activity of the molecule. Note that in SEQ ID NO:2 below, these cysteine residues appear as amino acids 96, 97, 329 and 330, the difference being the N-terminal methionine which is counted as residue 1 in the deduced sequence of SEQ ID:2 below and as residue 0 in the sequence of Lee et al. To remain consistent with prior work in the field, the numbering convention used by Lee et al. is used in this specification.

Please insert the following new paragraph for the paragraph which previously started on page 7, line 23 to page 7, line 36.

As will be discussed with the experimental results below, it was found possible to inhibit the formation of disulfide bonds between adjacent cysteine residues of a ribonuclease inhibitor by replacing the adjacent cysteine residues with alanine residues. The mutant human pancreatic ribonuclease inhibitor molecules thus created, have pairs of alanine-for-cysteine substitutions at both amino acids 94 and 95, at both amino acid positions [238] 328 and [239] 329, or substitutions for all four of the cysteine residues. It was demonstrated that the replacing of any or all of the cysteine residues with alanine did not markedly impair the ability of the human ribonuclease inhibitor to bind RNase A. There was, however, some slight diminution in affinity to ribonuclease for some of the variants.

In the Claims:

Please amend Claims 1 and 9 as follows and add the following new Claim 15:

1. A mutant ribonuclease inhibitor having at least one amino acid substitution in at least one of two [its] adjacent cysteine residues present in the amino acid sequence of the wild-type ribonuclease inhibitor, the substitution being to an amino acid residue not capable of forming a disulfide bond with an adjacent residue, the mutant ribonuclease inhibitor having a greater resistance to oxidation, the mutant ribonuclease inhibitor retaining its specificity and binding affinity to ribonuclease.

2. The ribonuclease inhibitor of claim 1, wherein ribonuclease inhibitor is a human ribonuclease inhibitor and the substituted cysteine residue is in at least one of positions 94, 95, 328 and 329.

3. The ribonuclease inhibitor of claim 1, wherein the cysteine residue is replaced with an alanine residue.

4. The ribonuclease inhibitor of claim 1, wherein the substitution in at least one of the cysteine residues inhibits the formation of a disulfide bond with an adjacent cysteine residue.

5. The ribonuclease inhibitor of claim 1, wherein the mutant ribonuclease inhibitor is 10 to 15 fold more resistant to oxidative damage than the native human ribonuclease inhibitor.

6. The ribonuclease inhibitor of claim 1, wherein the ribonuclease is of the RNASE A superfamily.

7. The ribonuclease inhibitor of claim 1, wherein the modified ribonuclease inhibitor exhibits an *in vitro* inhibition of ribonucleolytic activity.

8. The ribonuclease inhibitor of claim 1, wherein the mutant ribonuclease inhibitor is derived from the native human ribonuclease inhibitor.

9. A mutant human ribonuclease inhibitor having at least one amino acid substitution in at least one of [its] two adjacent cysteine residues present in the amino acid sequence of the wild-type ribonuclease inhibitor, the substitution being an amino acid other than cysteine, the mutant ribonuclease inhibitor having a greater resistance to oxidation, the mutant ribonuclease inhibitor retaining the specificity and binding affinity to angiogenin of the wild-type human ribonuclease inhibitor.

10. The ribonuclease inhibitor of claim 9, wherein the substituted cysteine residue is in at least one of positions 94, 95, 328 and 329.

11. A DNA sequence comprising a coding sequence encoding a mutant ribonuclease inhibitor which differs from the corresponding wild-type ribonuclease inhibitor in that at least one codon for cysteine has been replaced by a codon for another amino acid.

12. A DNA sequence as claimed in claim 11 wherein the replaced cysteine residue is adjacent to another cysteine residue in the wild-type sequence.

13. A DNA sequence as claimed in claim 11 wherein the ribonuclease inhibitor is human ribonuclease inhibitor and the cysteine replaced is at least one of amino acid positions 94, 95, 328 and 329.

14. A DNA sequence as claimed in claim 11 wherein the substitution is a codon for alanine.

15. (New) A mutant human ribonuclease inhibitor having at least one amino acid substitution in at least one of the amino acids positions 94, 95, 328 and 329, the substitution being an alanine for a cysteine, the mutant ribonuclease inhibitor having a greater resistance to oxidation, the mutant ribonuclease inhibitor retaining the specificity and binding affinity to angiogenin of the wild-type human ribonuclease inhibitor.